

PB86-213600

Toxicology of
Trans-1,2-Dichloroethylene in the Mouse

East Carolina Univ., Greenville, NC

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1985



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA <i>(Please read Instructions on the reverse before comp)</i>		
1. REPORT NO. EPA/600/J-85/417	2.	PB86-213600
4. TITLE AND SUBTITLE TOXICOLOGY OF <u>TRANS</u> -1,2-DICHLOROETHYLENE IN THE MOUSE (Journal Version)	5. REPORT DATE 1985	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Donald W. Barnes*, Virginia M. Sanders, Kimber L. White, Jr., George M. Shopp, Jr., and Albert E. Munson	8. PERFORMING ORGANIZATION REPORT NO.	
9. PERFORMING ORGANIZATION NAME AND ADDRESS *East Carolina University Greenville, NC 27834 Medical College of Virginia Richmond, VA 23298-0001	10. PROGRAM ELEMENT NO. CBMC1A	11. CONTRACT/GRANT NO. CR806481
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory (HERL) Office of Research and Development (ORD) U.S. Environmental Protection Agency (USEPA) Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED Journal Article	14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES P.O. Lyman Condie (for Campbell) TOTB/TMD/HERL/ORD/USEPA (CI) Citation: Drug and Chemical Toxicology, 8(5), 373-392. October 1985		
16. ABSTRACT Trans-1,2-dichloroethylene (DCE) was administered to male and female CD-1 mice in order to evaluate its effects on standard toxicological parameters. Following an acute LD50 determination (2122 mg/kg in males and 2391 mg/kg in females) and a 14-day range-finding study, a 90-day drinking water study was performed using levels of DCE calculated to deliver approximately 1/100, 1/10, and 1/5 the LD50. Various toxicological assessments were made, including body and organ weights, hematology, serum chemistries, and hepatic microsomal activities. Few alterations were observed in either sex following 90 days of exposure. The most noteworthy changes occurred in the males exposed to the highest level of DCE, where there was a significant decrease in glutathione levels, and in the females exposed to all three DCE levels, where there was a significant decrease in aniline hydroxylase activity. These data served as background for the immunotoxicological evaluation presented in the following manuscript.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 21
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

DRUG AND CHEMICAL TOXICOLOGY, 8(5), 373-392

TOXICOLOGY OF TRANS-1,2-DICHLOROETHYLENE IN THE MOUSE

Donald W. Barnes*, Virginia M. Sanders, Kimber L. White, Jr.,
George M. Shopp, Jr., and Albert E. Munson

*Department of Pharmacology
School of Medicine, East Carolina University
Greenville, NC 27834

and
Department of Pharmacology and Toxicology
Medical College of Virginia/Virginia Commonwealth University
Richmond, VA 23298-0001

ABSTRACT

Trans-1,2-dichloroethylene (DCE) was administered to male and female CD-1 mice in order to evaluate its effects on standard toxicological parameters. Following an acute LD50 determination (2122 mg/kg in males and 2391 mg/kg in females) and a 14-day range-finding study, a 90-day drinking water study was performed using levels of DCE calculated to deliver approximately 1/100, 1/10, and 1/5 the LD50. Various toxicological assessments were made, including body and organ weights, hematology, serum chemistries, and hepatic microsomal activities. Few alterations were observed in either sex following 90 days of exposure. The most noteworthy changes occurred in the males exposed to the highest level of DCE, where there was a significant decrease in glutathione levels, and in the females exposed to all three DCE levels, where there was a significant decrease in aniline hydroxylase activity. These data served as background for the immunotoxicological evaluation presented in the following manuscript.

INTRODUCTION

Dichloroethylenes exist in three isomeric forms: 1,1-dichloroethylene, cis-1,2-dichloroethylene, and trans-1,2-di-

chloroethylene (DCE). 1,1-Dichloroethylene is used as a chemical intermediate in the synthesis of methylchloroform and the manufacture of polyvinylidene chloride polymers, and is the only form of the dichloroethylenes used in large quantities for manufacturing and industrial purposes¹. However, all three isomers have been identified as contaminants in metropolitan drinking water². Although several studies have focused on the effects of 1,1-dichloroethylene,^{3,4} available toxicological data on DCE are limited. DCE, which is used as an industrial solvent, has been shown to be moderately toxic to a number of organ systems in experimental animals^{5,6}. These investigations found DCE, administered via the inhalation route, resulted in histological alterations in the hepatic, cardiac, and respiratory systems, in some instances even at the level of the current threshold limit value (TLV). Inhibition of the mixed function oxidase system also occurred at relatively low levels of exposure. This raises serious questions concerning the established TLV.

The purpose of this study was to evaluate the toxic effects of acute, 14- and 90-day oral exposure to DCE in the mouse. This manuscript examines several general toxicologically-related parameters while the following article⁷ describes the toxicity of DCE on the immune system.

MATERIALS AND METHODS

Details of the methods for the general toxicological assays and historical controls have been fully described by White et al. in the first article of this volume⁸.

Chemical

DCE, 98% pure, lot No. LC083187, was obtained from Aldrich Chemical Co., Milwaukee, WI. No stabilizers or preservatives were present with this chemical.

Chemical Administration

Mice received DCE by oral gavage during the acute and 14-day studies. DCE was prepared on the day of administration in a 1:9 (v/v) solution of emulphor (a polyethoxylated vegetable oil, EL 620, GAF Corp., New York, NY) and deionized water and maintained in dark glassware at 4°C until used. Preliminary studies done using various concentrations of emulphor demonstrated that DCE was maintained in solution while the emulphor vehicle caused no toxic effects in mice itself (data not shown). Concentrations of DCE were prepared such that the desired doses could be delivered in a volume of 0.01 ml/g of body weight by means of an 18 gauge feeding tube. In the acute study, DCE was administered as a single dose to male and female mice after an 18 hour fast. Nine doses ranging from 800–3500 mg/kg were used to generate dose response curves for DCE induced mortality. In the 14-day gavage study, concentrations of DCE were prepared so that each mouse received approximately 1/100 and 1/10 the LD50 (21 and 210 mg/kg) daily. In the 90-day study, DCE was administered ad libitum in the drinking water dissolved in deionized water containing 1% emulphor at either 0.1 mg/ml, 1.0 mg/ml, or 2.0 mg/ml. These concentrations were calculated to deliver approximately 1/100, 1/10, and 1/5 the LD50. Less than 10% of the DCE was lost during the three or four days between bottle changes, as measured by GLC with head space analysis. Twelve cages of control mice and eight cages of each treatment group per sex were used to estimate fluid consumption. DCE consumption was calculated from fluid consumption and is reported as mg DCE/kg/day. Fluid consumption is reported as ml/kg/day.

Hexobarbital Sleeping Time

Male and female mice were injected intravenously via the lateral tail vein with 100 mg/kg sodium hexobarbital (10 mg/ml) in saline. The duration of hexobarbital-induced narcosis was

determined by placing the anesthetized mice on their backs. Mice were considered awake when they were able to right themselves three times within a 20-second interval.

Statistical Evaluation

If a one-way analysis of variance of the means showed treatment effects, a Duncan's Multiple Range Test was performed⁹. Those values which differed from control at $p < 0.05$ were considered statistically significant. Each of the values represent the mean $\pm$ SE.

RESULTS

Acute Study

Table 1 shows the acute toxicity data for DCE. Animals were observed for 14 days, during which time no deaths occurred at doses up to 1200 mg/kg for males and females. The 3500 mg/kg dose was 100% lethal for males and 88% lethal for females. The LD50's with 95% confidence limits were 2122 (1874-2382) for male mice and 2391 mg/kg (2055-2788) for female mice.

Deaths occurred over a 10 day period following acute administration. Other responses observed were dependent on dose and time. Animals dosed at 1600, 2000, and 2400 mg/kg demonstrated signs of decreased activity and ruffled fur within two hours after gavage. There was a higher incidence of these signs of toxicity in the males than in the females. In the higher dose groups there were signs of ataxia, suppressed or total loss of the righting reflex, and ruffled fur. Animals in the 2800 mg/kg groups exhibited ruffled fur for the three days following exposure. At 3000 and 3500 mg/kg all animals had ruffled fur and a hunch-backed appearance until death. Necropsies were performed

TABLE 1
Acute Toxicity (mg/kg) of DCE in CD-1 Mice^a

Sex	LD10	LD50	LD90	Slope	No. Mice
Male	1378 (1007-1615)	2122 (1874-2382)	3298 (2820-4344)	1.76	92
Female	1533 (978-1840)	2391 (2055-2788)	3728 (3101-5885)	2.67	63

^aCD-1 male and female mice were fasted for 18 hr prior to a single gavage of DCE in nine doses ranging between 800 mg/kg and 3500 mg/kg. The LD10, LD50, and LD90 with corresponding 95% confidence limits (in parentheses) and the slope were calculated according to the Log Probit Procedure¹⁰.

on all dead animals, and revealed evidence of hyperemia of mucosal surface of the stomach and small intestines.

Fourteen Day Study

No significant differences existed among the groups with respect to weight gain over the 14-day exposure period (data not shown). Weights of the brain, liver, spleen, lungs, thymus, kidney, and testes were not altered by DCE exposure (Table 2). All organ weights were within the limits of our historical controls and there was no treatment effect when the weights were expressed either as absolute weight, percent of body weight, or as organ to brain ratios (data not shown).

The effect of DCE on certain hematological parameters are shown in Table 3. No changes were seen in hematocrit or hemoglobin values. Fibrinogen levels were decreased (12%) in the 210 mg/kg treatment group, while prothrombin activity increased slightly as manifested by a 7% decrease in prothrombin time. Serum enzyme and blood urea nitrogen levels of the three experimental groups are shown in Table 4. No significant differences

TABLE 2
Body and Organ Weights of Male CD-1 Mice Exposed for
Fourteen Days by Gavage to DCE^a

Parameter	Vehicle ^b	21 mg/kg DCE	210 mg/kg DCE
Body Weight (g)	30.7 ± 0.6	31.5 ± 0.5	31.0 ± 1.0
Brain (mg) (% body weight)	485 ± 9 (1.59)	486 ± 15 (1.54)	490 ± 11 (1.59)
Liver (mg) (% body weight)	1830 ± 75 (5.96)	1835 ± 67 (5.81)	1761 ± 80 (5.66)
Spleen (mg) (% body weight)	171 ± 12 (0.56)	159 ± 16 (0.50)	160 ± 11 (0.51)
Lungs (mg) (% body weight)	240 ± 9 (0.78)	226 ± 9 (0.72)	226 ± 8 (0.73)
Thymus (mg) (% body weight)	94 ± 6 (0.31)	78 ± 7 (0.25)	88 ± 6 (0.28)
Kidneys (mg) (% body weight)	552 ± 19 (1.80)	549 ± 24 (1.74)	556 ± 26 (1.79)
Testes (mg) (% body weight)	245 ± 12 (0.80)	229 ± 9 (0.73)	249 ± 15 (0.80)

^aValues represent mean ± SE derived from 9-10 mice per group.
^bVehicle = 10% emulphor in deionized water.

occurred in the SGPT and BUN levels. However, there was a significant decrease (29%) in LDH levels of the group treated with 210 mg/kg DCE.

Ninety Day Study

Since the toxicity observed in the 14-day study was minimal, the concentrations of DCE presented to the mice in the 90-day drinking water study were calculated so that the total daily

TABLE 3
Hematological and Coagulation Values for Male CD-1 Mice
Exposed for Fourteen Days by Gavage to DCE^a

Parameter	Vehicle ^b	21 mg/kg DCE	210 mg/kg DCE
Hematocrit (%)	42.4 ± 0.7	41.8 ± 0.7	41.7 ± 0.6
Hemoglobin (g%)	16.4 ± 0.4	16.4 ± 0.5	16.2 ± 0.2
Leukocytes (x 10 ³ /mm ³)	10.06 ± 1.02	8.66 ± 1.17	7.44 ± 0.53
Fibrinogen (mg%)	372 ± 12	373 ± 15	327 ± 13*
Prothrombin Time (seconds)	6.9 ± 0.1	6.8 ± 0.0	6.4 ± 0.1*

^aValues represent mean ± SE derived from 9-10 mice per group.
Those values which differ from vehicle control at $p < 0.05$ are noted by an asterisk.

^bVehicle = 10% emulphor in deionized water.

TABLE 4
Serum Enzyme and Urea Nitrogen Levels in Male CD-1 Mice
Exposed for Fourteen Days to DCE^a

Exposure Group	LDH (IU/L)	SGPT (IU/L)	BUN (IU/L)
Vehicle	1007 ± 100	36.7 ± 6.3	24.1 ± 1.3
21 mg/kg	810 ± 38	28.2 ± 2.1	25.0 ± 1.2
210 mg/kg	716 ± 44*	27.1 ± 1.3	21.9 ± 1.0

^aValues represent mean ± SE derived from 9-10 mice per group.
Those values which differ from vehicle control (10% emulphor in deionized water) at $p < 0.05$ are noted by an asterisk. LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; BUN = blood urea nitrogen

TABLE 5
Time-Weighted Averages of Fluid Consumption by CD-1 Mice
Receiving DCE in the Drinking Water for 90 Days^a

Concen- tration	DAYS OF THE STUDY ^b			CUMULATIVE (DAYS 0-90)		
	(mg/ml) 0-30	31-60	61-90	ml/kg/day	mg/kg/day ^c	ml/mouse/day ^d
M A L E S						
0	194 ± 2	165 ± 2	147 ± 2	170 ± 3	0	5.9 ± 0.1
0.1	188 ± 3	165 ± 2	151 ± 2	168 ± 3	16.8 ± 0.3	6.0 ± 0.1
1.0	198 ± 3	170 ± 4	154 ± 2	175 ± 5	175 ± 5	6.3 ± 0.2
2.0	216 ± 5	196 ± 4	165 ± 3	193 ± 8	387 ± 16	6.9 ± 0.2
F E M A L E S						
0	230 ± 2	214 ± 3	203 ± 3	217 ± 5	0	6.3 ± 0.1
0.1	243 ± 3	218 ± 4	219 ± 3	226 ± 4	22.6 ± 0.4	6.6 ± 0.1
1.0	251 ± 3	221 ± 5	201 ± 3	224 ± 6	224 ± 6	6.6 ± 0.2
2.0	244 ± 2	234 ± 3	199 ± 2	226 ± 5	452 ± 9	6.6 ± 0.2

^aValues represent mean ± SE derived from 48 mice in control (deionized H₂O) group and 31-32 mice in the other groups. Means were calculated for each month of study and the entire 90 days.

^bAverage ml fluid/kg body weight/day for the period indicated.

^cAverage mg of DCE per body weight/day.

^dAverage ml fluid/mouse/day.

intake was comparable to, and higher than, the doses used in the 14-day study (i.e., approximately 1/100, 1/10, and 1/5 the LD₅₀). The concentrations of DCE delivered in the drinking water were 0.1, 1.0, and 2.0 mg/ml. In addition, a group receiving deionized water served as controls. There were 260 mice of each sex in the control group and 140 mice of each sex in each treatment group. Table 5 summarizes the time weighted averages of fluid and DCE consumption in both male and female mice at 30-day intervals. On a ml/kg basis, fluid consumption by both sexes progressively decreased throughout the duration of the experiment, with comparable changes occurring in the control group. The average doses of DCE consumed on a mg/kg/day basis over the

TABLE 6
Body and Organ Weights of Male CD-1 Mice Exposed to DCE
in the Drinking Water for 90 Days^a

Parameter	Deionized Water	DCE		
		0.1 mg/ml	1.0 mg/ml	2.0 mg/ml
Body Wt. (g)	39.8 ± 0.7	40.1 ± 1.0	41.4 ± 0.9	38.9 ± 0.9
Brain (mg)	533 ± 5	531 ± 6	527 ± 9	520 ± 6
(% body wt.)	(1.35)	(1.34)	(1.28)	(1.35)
Liver (mg)	2029 ± 43	2007 ± 62	2288 ± 60*	2022 ± 85
(% body wt.)	(5.10)	(5.01)	(5.53*)	(5.17)
Spleen (mg)	146 ± 6	130 ± 8	145 ± 7	133 ± 9
(% body wt.)	(0.37)	(0.32)	(0.35)	(0.34)
Lungs (mg)	232 ± 4	228 ± 5	236 ± 6	223 ± 5
(% body wt.)	(0.58)	(0.57)	(0.57)	(0.58)
Thymus (mg)	48 ± 3	47 ± 3	54 ± 2	48 ± 2
(% body wt.)	(0.12)	(0.12)	(0.13)	(0.12)
Kidneys (mg)	651 ± 21	637 ± 22	658 ± 19	634 ± 27
(% body wt.)	(1.64)	(1.59)	(1.59)	(1.63)
Testes (mg)	265 ± 8	270 ± 8	263 ± 6	248 ± 10
(% body wt.)	(0.67)	(0.68)	(0.64)	(0.64)

^aValues represent mean ± SE derived from 23 mice in the control group and 15-16 mice in the other groups. Values which differ significantly from control at $p < 0.05$ are noted by an asterisk.

90 day period were 17, 175, and 387 for the males, and 23, 224, and 452 for the females.

No DCE-induced changes in terminal body weight or gross pathology were observed at the time of necropsy in either sex. However, on a percent of body weight basis, male mice receiving 1.0 mg/ml DCE demonstrated an 8% increase in liver weights

TABLE 7
Body and Organ Weights of Female CD-1 Mice Exposed to DCE
in the Drinking Water for 90 Days^a

Parameter	Deionized H2O	0.1 mg/ml	DCE	
			1.0 mg/ml	2.0 mg/ml
Body Wt. (g)	32.4 ± 0.6	33.8 ± 0.7	34.0 ± 0.6	31.8 ± 0.6
Brain (mg)	472 ± 7	481 ± 8	484 ± 11	487 ± 6
(% body wt.)	(1.47)	(1.43)	(1.43)	(1.54)
Liver (mg)	1712 ± 57	1839 ± 51	1864 ± 38	1741 ± 57
(% body wt.)	(5.27)	(5.44)	(5.49)	(5.46)
Spleen (mg)	198 ± 11	192 ± 12	196 ± 9	190 ± 11
(% body wt.)	(0.61)	(0.57)	(0.58)	(0.60)
Lungs (mg)	254 ± 11	255 ± 7	244 ± 7	222 ± 8*
(% body wt.)	(0.79)	(0.76)	(0.72)	(0.70*)
Thymus (mg)	71 ± 3	67 ± 4	61 ± 4	54 ± 4*
(% body wt.)	(0.22)	(0.20)	(0.18*)	(0.17*)
Kidneys (mg)	461 ± 12	456 ± 9	465 ± 13	428 ± 8
(% body wt.)	(1.43)	(1.35)	(1.37)	(1.35)

^aValues represent mean ± SE derived from 24 mice in the de-ionized water group and 16 mice in the other groups. Values which differ significantly from control at $p < 0.05$ are noted by an asterisk.

(Table 6), while female mice exposed to 2.0 mg/ml DCE demonstrated an 11% decrease in lung weight (Table 7). In addition, thymus weights were decreased 18 and 33% in female mice exposed to 1.0 and 2.0 mg/ml DCE (Table 7).

Hematological findings are summarized in Tables 8, 9, and 10. Exposure to DCE produced no remarkable changes in these parameters, although prothrombin time was significantly decreased by 15% and 12% in male mice exposed to 0.1 and 1.0 mg/

353

TABLE 8
Hematology of Male CD-1 Mice Exposed to DCE in the Drinking
Water for 90 Days^a

Parameter	Deionized Water	DCE		
		0.1 mg/ml	1.0 mg/ml	2.0 mg/ml
Hematocrit (%)	40.3 ± 0.5	40.0 ± 0.5	41.1 ± 0.8	41.9 ± 0.4
Hemoglobin (g%)	12.8 ± 0.2	12.7 ± 0.2	13.1 ± 0.3	12.9 ± 0.2
Erythrocytes (10 ⁶ /mm ³)	7.80 ± 0.20	7.56 ± 0.28	8.01 ± 0.20	8.35 ± 0.22
Leukocytes (10 ³ /mm ³)	5.30 ± 0.32	4.95 ± 0.42	4.83 ± 0.24	5.16 ± 0.40
Platelets (10 ⁵ /mm ³)	3.81 ± 0.16	3.96 ± 0.15	3.63 ± 0.14	3.64 ± 0.13
Fibrinogen (mg%)	259 ± 8	256 ± 17	259 ± 10	268 ± 9
Prothrombin Time (sec)	10.0 ± 0.2	8.5 ± 0.2*	8.8 ± 0.3*	9.8 ± 0.2

^aValues represent mean ± SE derived from 23 mice in control group and 15-16 mice in other groups. Values which differ significantly from control at $p < 0.05$ are noted by an asterisk.

TABLE 9
Hematology of Female CD-1 Mice Exposed to DCE in the Drinking
Water for 90 Days^a

Parameter	Deionized Water	DCE		
		0.1 mg/ml	1.0 mg/ml	2.0 mg/ml
Hematocrit (%)	41.9 ± 0.3	42.2 ± 0.7	42.9 ± 0.6	42.1 ± 0.3
Hemoglobin (g%)	13.4 ± 0.1	13.2 ± 0.2	13.4 ± 0.2	13.1 ± 0.2
Erythrocytes (10 ⁶ /mm ³)	8.46 ± 0.16	8.25 ± 0.14	8.15 ± 0.44	8.66 ± 0.19
Leukocytes (10 ³ /mm ³)	7.27 ± 0.32	6.98 ± 0.50	8.95 ± 0.61*	7.79 ± 0.64
Platelets (10 ⁵ /mm ³)	3.71 ± 0.10	3.78 ± 0.13	3.59 ± 0.11	3.66 ± 0.12
Fibrinogen (mg%)	199 ± 7	199 ± 8	218 ± 7	204 ± 4
Prothrombin Time (sec)	9.7 ± 0.2	9.8 ± 0.3	9.1 ± 0.2	9.0 ± 0.6

^aValues represent mean ± SE derived from 23 mice in control group and 15-16 mice in other groups. Values which differ significantly from control at $p < 0.05$ are noted by an asterisk.

TABLE 10
Differential Cell Counts of Mice Exposed to DCE in the
Drinking Water for 90 Days^a

Exposure Group	Percent of Total White Blood Cells			
	Lymphocytes	Polymorphonuclears	Monocytes	Eosinophils
<u>M A L E S</u>				
Control	72.9 ± 3.2	24.4 ± 3.3	1.8 ± 0.6	0.9 ± 0.3
0.1 mg/ml	72.5 ± 3.1	24.9 ± 2.7	2.1 ± 0.9	0.5 ± 0.3
1.0 mg/ml	67.8 ± 4.2	27.8 ± 4.2	3.1 ± 1.0	1.4 ± 0.5
2.0 mg/ml	63.1 ± 4.4	33.8 ± 4.2	2.4 ± 0.8	0.7 ± 0.4
<u>F E M A L E S</u>				
Control	76.3 ± 2.5	17.3 ± 2.1	5.7 ± 0.8	0.7 ± 0.3
0.1 mg/ml	78.4 ± 2.5	18.1 ± 2.6	3.2 ± 1.0	0.3 ± 0.2
1.0 mg/ml	83.2 ± 1.9	10.0 ± 1.5*	4.2 ± 0.6	2.5 ± 0.6*
2.0 mg/ml	72.4 ± 2.4	23.6 ± 2.1	3.2 ± 0.7*	0.8 ± 0.3

^aValues represent mean ± SE derived from 8-15 mice per group.
Values which differ significantly from control (deionized water) at $p < 0.05$ are noted by an asterisk.

ml, respectively. A slight increase (23%) in the number of blood leukocytes occurred in female mice exposed to 1.0 mg/ml DCE. A differential analysis of the white cells from this group of females revealed a slight increase in the percent of lymphocytes (not significant) and a significant decrease (42%) in the percent of polymorphonuclear leukocytes.

Exposure to DCE produced slight changes in several clinical chemistry parameters in males (Table 11) and females (Table 12). Although these values were significantly different from those of

TABLE 11
Serum and Liver Chemistry Values of Male CD-1 Mice
Exposed to DCE in the Drinking Water for 90 Days^a

Parameter	Deionized Water	DCE		
		0.1 mg/ml	1.0 mg/ml	2.0 mg/ml
Calcium (mg%)	10.1 ± 0.1	9.8 ± 0.1	9.7 ± 0.2	10.4 ± 0.3
Sodium (mEq/L)	156 ± 1	157 ± 1	153 ± 1*	152 ± 1*
Chloride (mEq/L)	104 ± 3	107 ± 3	111 ± 2	111 ± 5
Potassium (mEq/L)	7.62 ± 0.12	8.26 ± 0.18*	8.11 ± 0.16*	8.02 ± 0.20
Protein (g%)	6.23 ± 0.07	6.56 ± 0.11*	6.19 ± 0.09	6.19 ± 0.12
Glucose (mg%)	153 ± 7	195 ± 8*	184 ± 5*	190 ± 7*
Cholesterol (mg%)	137 ± 5	144 ± 7	126 ± 4	130 ± 4
BUN (mg%)	22.8 ± 1.0	22.5 ± 1.1	25.1 ± 1.1	23.4 ± 1.6
LDH (IU/L)	677 ± 33	605 ± 47	449 ± 22*	587 ± 56
SGPT (IU/L)	44.3 ± 3.3	55.1 ± 7.1	45.0 ± 6.8	41.2 ± 4.7
SGOT (IU/L)	74.0 ± 6.5	110.0 ± 7.8*	65.3 ± 5.0	69.9 ± 5.8
SAP (IU/L)	34.3 ± 1.8	37.6 ± 5.1	55.5 ± 5.4*	45.6 ± 2.4*
Creatinine (mg%)	0.64 ± 0.04	0.60 ± 0.04	0.61 ± 0.04	0.62 ± 0.02
Glutathione (μmol/g liver)	8.38 ± 0.31	8.04 ± 0.22	8.90 ± 0.33	6.61 ± 0.19*

^aValues represent mean ± SE derived from 24 mice in the de-ionized water group and 16 mice in other groups. Values which differ significantly from control at $p < 0.05$ are noted by an asterisk. BUN = blood urea nitrogen; LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxaloacetic transaminase; SAP = serum alkaline phosphatase.

TABLE 12
Serum and Liver Chemistry Values of Female CD-1 Mice
Exposed to DCE in the Drinking Water for 90 Days^a

Parameter	Deionized Water	0.1 mg/ml	DCE	
			1.0 mg/ml	2.0 mg/ml
Calcium (mg%)	10.7 ± 0.1	10.4 ± 0.2	9.7 ± 0.3*	9.4 ± 0.2*
Sodium (mEq/L)	150 ± 1	150 ± 2	152 ± 1	154 ± 1
Chloride (mEq/L)	102 ± 3	104 ± 4	105 ± 2	103 ± 3
Potassium (mEq/L)	6.21 ± 0.12	6.65 ± 0.19*	7.29 ± 0.17*	6.83 ± 0.17*
Protein (g%)	7.38 ± 0.09	7.30 ± 0.16	7.14 ± 0.11	7.09 ± 0.11
Glucose (mg%)	122 ± 3	156 ± 6*	147 ± 5*	156 ± 6*
Cholesterol (mg%)	85.4 ± 2.7	86.4 ± 3.0	88.1 ± 3.3	88.2 ± 4.9
BUN (mg%)	20.3 ± 0.4	21.5 ± 0.7	18.6 ± 0.8	18.8 ± 0.9
LDH (IU/L)	511 ± 22	377 ± 20*	452 ± 23	559 ± 42
SGPT (IU/L)	49.9 ± 6.4	38.3 ± 3.0	33.5 ± 3.6*	30.4 ± 1.6*
SGOT (IU/L)	91.7 ± 6.6	77.8 ± 6.0	66.9 ± 4.5*	58.9 ± 8.8*
SAP (IU/L)	44.0 ± 2.3	47.6 ± 4.2	51.0 ± 3.0	45.4 ± 2.9
Creatinine (mg%)	0.75 ± 0.04	0.75 ± 0.04	0.68 ± 0.04	0.82 ± 0.03
Glutathione (μmol/g liver)	8.53 ± 0.28	8.29 ± 0.24	8.23 ± 0.21	8.25 ± 0.28

^aValues represent mean ± SE derived from 24 mice in the de-ionized water group and 14-16 mice in other groups. Values which differ significantly from control at $p < 0.05$ are noted by an asterisk. BUN = blood urea nitrogen; LDH = lactate dehydrogenase; SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic-oxaloacetic transaminase; SAP = serum alkaline phosphatase.

TABLE 13
Hexobarbital Sleeping Times of CD-1 Mice Exposed to DCE
in the Drinking Water for 90 Days^a

Exposure Group	MALES Sleep Time (min)	FEMALES Sleep Time (min)
Deionized Water	38.9 ± 3.7	37.6 ± 5.6
0.1 mg/ml	30.5 ± 3.9	51.4 ± 4.8
1.0 mg/ml	30.5 ± 2.9	47.8 ± 4.9
2.0 mg/ml	33.8 ± 4.9	43.9 ± 3.0

^aValues represent mean ± SE derived from 7 mice in control (deionized water) group and 8 mice in other groups.

the controls, there were no consistent trends or any large deviations from historical control values. The most noteworthy changes were in the glucose levels of both sexes, which were elevated at all three dose levels, and in the glutathione levels of males exposed to 2.0 mg/ml, which were decreased 21% below control. Also, SGPT and SGOT levels were decreased at all dose levels in the females.

The effects of DCE exposure on hepatic microsomal drug metabolism abilities were assessed by measuring hexobarbital sleeping times and by quantitating mixed function oxidases in microsomes from livers of male and female mice. DCE exposure had no effect on the duration of hexobarbital-induced narcosis in either sex (Table 13). In male mice (Table 14), exposure to 1.0 mg/ml DCE resulted in decreases in the activities of aminopyrine N-demethylase (17%) and aniline hydroxylase (27%). In contrast, no significant changes in these enzyme activities were observed in the 2.0 mg/ml exposure group in males. Microsomal protein and cytochrome P-450 and b5 content were not affected by exposure to

TABLE 14
Hepatic Microsomal Activities in Male CD-1 Mice Exposed to
DCE in the Drinking Water for 90 Days^a

Parameter	Deionized Water	DCE		
		0.1 mg/ml	1.0 mg/ml	2.0 mg/ml
Microsomal Protein (mg/g liver)	21.9 ± 0.5	22.0 ± 0.4	22.6 ± 0.6	21.1 ± 0.8
Cytochrome P-450 (nmol/mg protein)	0.982 ± .034	1.034 ± .046	0.897 ± .045	0.925 ± .056
Cytochrome b5 (nmol/mg protein)	0.377 ± .014	0.382 ± .010	0.368 ± .016	0.377 ± .019
Aminopyrine N-demethylase (nmol/mg/min)	10.77 ± 0.27	10.43 ± 0.36	8.95 ± 0.38*	9.65 ± 0.68
Aniline Hydroxylase (nmol/mg/min)	1.119 ± .083	1.084 ± .093	0.875 ± .082*	1.004 ± .087

^aValues represent mean ± SE derived from 8 mice per group. Values which differ significantly from control (deionized water) at $p < 0.05$ are noted by an asterisk.

DCE. In female mice (Table 15), microsomal protein content was increased (16%) in the group exposed to 0.1 mg/ml, while no changes were observed in the other groups. Microsomal cytochrome P-450 and b5 contents in all DCE exposed groups were similar to control values. On the other hand, aniline hydroxylase activity was significantly depressed in all exposure groups, although the decreases (21%, 33%, and 28%) did not appear to be dose-dependent. Aminopyrine N-demethylase activity was slightly decreased (11%) in the 1.0 mg/ml group.

DISCUSSION

The toxic effects of oral exposure to the dichloroethylenes, particularly the 1,2-isomers, are not well described. The

TABLE 15
Hepatic Microsomal Activities in Female CD-1 Mice Exposed to
DCE in the Drinking Water for 90 Days^a

Parameter	Deionized Water	0.1 mg/ml	DCE	
			1.0 mg/ml	2.0 mg/ml
Microsomal Protein (mg/g liver)	18.8 ± 0.6	21.8 ± 0.8*	19.0 ± 0.3	18.6 ± 0.6
Cytochrome P-450 (nmol/mg protein)	0.864 ± .060	0.919 ± .032	0.844 ± .025	0.832 ± .021
Cytochrome b5 (nmol/mg protein)	0.492 ± .014	0.500 ± .017	0.522 ± .012	0.522 ± .018
Aminopyrine N-demethylase (nmol/mg/min)	11.75 ± 0.46	10.91 ± 0.40	10.42 ± 0.20*	10.64 ± 0.32
Aniline Hydroxylase (nmol/mg/min)	1.813 ± .054	1.426 ± .041*	1.224 ± .033*	1.309 ± .056*

^aValues represent mean ± SE derived from 8 mice per group.
Values which differ significantly from control (deionized water)
at $p < 0.05$ are noted by an asterisk.

present studies of the toxicity of DCE, administered by gavage or in drinking water, were conducted because of its presence in human drinking water supplies². Acute, 14-, and 90-day studies were performed. The acute lethal effects of DCE appear to be related to suppression of the central nervous system. The LD50's for DCE for male (2122 mg/kg) and female (2391 mg/kg) mice are more than ten times greater than those reported for 1,1-dichloroethylene (217 mg/kg and 194 mg/kg)³. There appears to be no sex-related difference in the LD50's for either isomer.

Since the LD50's were similar in both sexes, a 14-day study was performed in male mice in order to assess cumulative toxicity and provide preliminary data for the design of the 90-day

drinking water studies. DCE was administered by daily oral gavage at single doses of 1/10 and 1/100 the acute LD50. No remarkable exposure-related effects were apparent at either dose.

In light of the low toxicity observed in the 14-day study, the 90-day drinking water study was conducted with concentrations of DCE that would deliver daily doses comparable to those in the 14-day study, plus an additional higher concentration (1/5 the LD50). After 90 days of exposure to DCE, the toxic effects of DCE appeared to be minimal, as judged by evaluation of gross pathological, hematological, and clinical chemistry parameters.

Previous reports have established that the symmetrically chlorinated ethylenes, such as DCE, are not mutagenic⁴. The isomer of DCE, 1,1-dichloroethylene, has been demonstrated to be nephrotoxic, hepatotoxic, and mutagenic^{4,11,12} as a result of its conversion to reactive metabolites^{13,14}. At the concentrations employed in the 14- and 90-day studies, DCE appeared to have little or no effect on renal function, as judged by creatinine and BUN levels, or hepatic function, as judged by serum enzyme and protein levels.

Exposure of rats to 200, 600, or 1000 ppm in air to cis-1,2-dichloroethylene and DCE for 8 hr produced dose-dependent increases in both hexobarbital sleeping time and zoxazolamine paralysis time⁶, with cis-1,2-dichloroethylene producing a greater degree of inhibition than DCE. These authors also demonstrated that DCE competitively inhibited the O-demethylation of p-nitroanisole and the N-demethylation of aminopyrine by hepatic microsomes. In the present study, effects of DCE on the functional integrity of microsomal mixed function oxidase components were assessed after 90 days of oral exposure. In both male and female mice, DCE had no effect on microsomal cytochrome P-450 and b5 content. However, in female mice, aniline hydroxylase activity was significantly depressed at all treatment levels.

At the 2.0 mg/ml level, hepatic glutathione was significantly decreased in male mice; however, this change was not associated with any changes in liver or microsomal function.

In conclusion, the most notable effects of oral exposure to DCE were decreased glutathione levels in males and decreased aniline hydroxylase activity in females.

ACKNOWLEDGEMENTS

This work was supported by grants from the Environmental Protection Agency (R806481010) and the National Institute of Environmental Health Sciences (1T32ES07087).

REFERENCES

1. D.D. Irish, Halogenated hydrocarbons: I. Aliphatic, in Industrial Hygiene and Toxicology, vol. 2, F.A. Patty, ed., John Wiley and Sons, New York, 1963, p. 1305.
2. United States Environmental Protection Agency, Preliminary assessment of suspected carcinogens in drinking water. Report to Congress, Office of Toxic Substances, USEPA, Washington, DC, 1975.
3. B.K. Jones and D.E. Hathway, Differences in metabolism of vinylidene chloride between mice and rats, Br. J. Cancer., 37, 411 (1978).
4. E.S. Reynolds and M.T. Moslen, Damage to hepatocellular membranes by chlorinated olefins with emphasis on synergism and antagonism, Environ. Health Persp., 21, 137 (1977).
5. K.J. Frendt, G.P. Liebaladt, and E. Lieberwirth, Toxicity studies on trans-1,2-dichloroethylene, Toxicology, 7, 141 (1979).
6. K.J. Frendt and J. Macholz, Inhalation of mixed function oxidases in rat liver by trans- and cis-1,2-dichloroethylene, Toxicology, 10, 131 (1978).
7. G.M. Shopp, Jr., V.M. Sanders, K.L. White, Jr., L.E. Sain, R.A. Carchman, J.F. Borzelleca, and A.E. Munson, Humoral

- and cell-mediated immune status of mice exposed to trans-1,2-dichloroethylene, Drug Chem. Toxicol. _____ (1985).
8. K.L. White, Jr., V.M. Sanders, D.W. Barnes, L.E. Sain, G.M. Shopp, Jr., R.A. Carchman, J.F. Borzelleca, and A.E. Munson, Immunotoxicological investigations in the mouse. General approach and methods, Drug Chem. Toxicol. _____ (1985).
 9. J.P. Sall, Duncan Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute, Inc., Raleigh, NC, 1979, p. 191.
 10. J.H. Goodnight, Probit Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute, Inc., Raleigh, NC, 1979, p. 357.
 11. M.E. Andersen, J.E. French, M.L. Gargas, R.A. Jones, and L. J. Jenkins, Saturable metabolism and acute toxicity of 1,1-dichloroethylene, Toxicol. Appl. Pharmacol., 47, 385 (1979).
 12. C.P. Siegers, M. Younes, and G. Schmitt, Effects of dithiocarb and (+)-cyanidol-3 on the hepatotoxicity and metabolism of vinylidene chloride in rats, Toxicology, 15, 55 (1979).
 13. M.E. Andersen and L.J. Jenkins, The oral toxicity of 1,1-dichloroethylene in the rats. Effects of sex, age, and fasting, Environ. Health Persp., 21, 157 (1977).
 14. M.J. McKenna, P.G. Watanabe, and P.J. Gehring, Pharmacokinetics of vinylidene chloride in the rat, Environ. Health Persp., 21, 91 (1977).

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.